Evaluation of anthelmintic resistance in Oklahoma beef cattle herds and assessment of composite sampling for herd level fecal egg count reduction testing

John N. Gilliam,[1] DVM, MS, DACVIM, DABVP; **Jared D. Taylor**,[2] DVM, MPH, PhD, DACVIM (LA), DACVPM; **Ruth Scimeca**,[2] VMD, MSc, PhD, DACVM

[1]Department of Veterinary Clinical Sciences, Oklahoma State University, College of Veterinary Medicine, Stillwater, OK 74078
[2]Department of Veterinary Pathobiology, Oklahoma State University, College of Veterinary Medicine, Stillwater, OK 74078
Corresponding author: Dr. John Gilliam, john.gilliam@okstate.edu

Abstract

Beef cow-calf producers submitted fecal samples for fecal egg count reduction (FECR) tests. Anthelmintic administration practices were not controlled, and producers were encouraged to follow standard procedures for each herd. Fecal egg counts (FEC) were determined using the Wisconsin method with a limit of detection of 1 egg per gram (EPG). Inclusion criteria included a pretreatment FEC of 25 EPG. A FECR of ≤ 90% was considered indicative of resistance. Seventeen herds submitted a total of 19 sample sets. All major classes of anthelmintics were represented except for levamisole. Injectable, pour-on and oral as well as original label and generic products were represented. Sample sets from 3 herds were excluded from the final analysis based on inadequate pretreatment FEC. Of the 16 sample sets included in the final analysis, 13 exhibited resistance based on the arithmetic mean of individual FECR tests (FECRT). In addition to individual FEC, composite samples for each herd were created using 1 gram of feces from each animal. Four grams of each composite sample were used to determine a composite FEC. Composite samples from 13 herds were included in the final analysis and 11 of 13 exhibited resistance. There was complete agreement between the individual and composite samples for the detection of resistance. Based on the results of this small survey, apparent anthelmintic resistance appears to be widespread in beef cow-calf herds in Oklahoma. Composite sampling appears to be an effective method for detection of herd level resistance.

Key words: anthelmintic resistance, beef cattle, FECR, composite

Introduction

Gastrointestinal nematodes represent a major challenge to grazing livestock production throughout the world. These parasites can cause a variety of clinical signs including weight loss, diarrhea and even death in some situations. However, subclinical disease in the form of production loss is more common and more important economically. Treatment of cattle for subclinical parasitism has been found to result in improved growth in beef calves and improved reproductive performance in beef cows.[1-4] Given these benefits and with the widespread availability of effective anthelmintics labeled for cattle, it is typical for cattle producers to routinely deworm cattle. According to the 2007-2008 National Animal Health Monitoring System (NAHMS) Beef Cow-Calf Survey[a], over 50% of beef producers deworm preweaned calves at least once per year while over 80% deworm cows at least once per year. The widespread use of anthelmintics has raised concerns about the development of parasite resistance leading to loss of efficacy of anthelmintic products. Gastrointestinal nematode resistance in stocker cattle was first identified in the United States in 2003 and reported in 2009.[5] Resistance to macrocyclic lactones and benzimidazoles was confirmed the following year by the same research group.[6] Cattle in both of these studies originated in the southeastern United States and were grazed in Wisconsin at the time resistance was detected. In 2010, Edmonds et al. reported resistance to macrocyclic lactones in *Ostertagia ostertagi* and *Cooperia oncophora* in cattle originating from Northern Californina.[7] Taken together, these studies demonstrate that anthelmintic resistance is not isolated to a particular geographic region. As part of the 2007-2008 NAHMS Beef Cow-Calf Survey, producers were given the opportunity to submit fecal samples to investigate the prevalence of resistance.[8] In this study, fecal egg count reduction tests (FECRT) indicating efficacy of less than 90% were considered indicative of resistance. Four out of 12 herds representing the southeastern U.S. had evidence of resistance, including 2 of 4 herds from Oklahoma. To our knowledge, this study is the only report of anthelmintic resistance in Oklahoma beef cattle herds, but the relatively small size and nature of participant selection limit the representativeness of the results. Because Oklahoma ranks second in the nation for beef cow numbers, a good understanding of the prevalence of anthelmintic resistance is important to maintenance of optimal production and the creation of effective parasite control programs that minimize further development of resistance.

Data regarding beef producers' use of laboratory testing to assess anthelmintic efficacy is not available, but the frequency is thought to be very low. Necropsy of representative animals to allow direct counts of abomasal and intestinal worms is the gold standard for assessment of worm burdens and anthelmintic efficacy. However, this methodology is impractical for use in commercial production settings. The FECRT[9] is the most practical method of assessing anthelmintic efficacy for livestock producers. Based on NAHMS 2007-2008 survey data, less than 1 percent of beef producers utilize laboratory testing to determine optimal timing of anthelmintic administration[a].

The cost of repeated fecal egg counts (FEC) on multiple animals likely prevents many producers from assessing the efficacy of anthelmintic control programs. Testing composite, or pooled, samples rather than individual animals could be a way to evaluate resistance at the herd level while reducing the expense and encouraging more producers to assess the efficacy of their anthelmintic programs. The evaluation of composite fecal samples for fecal egg count reduction testing in sheep[10,11] and cattle[12] has been previously described.

The primary purpose of this study was to estimate the prevalence of anthelmintic resistance in beef cow-calf herds in the state of Oklahoma and a secondary objective was to evaluate the use of composite fecal samples for the detection of anthelmintic resistance at the herd level.

Materials and methods

Animals and sample collection

Participating herds were identified through cooperating veterinarians and extension personnel. Selection criteria for herds was simply the willingness to participate in the study, and the only criteria for participating were that the herd was located in Oklahoma, calves would be individually identified, and that the same calves would be sampled pre and post-treatment. The target number for participating herds was 20. A target of 20 herds was thought to be large enough to be representative of the industry in the state and still fit within the labor and budget constraints of the study. Sample collection occurred in 2020. One group of calves consisted of yearling calves weaned in the fall of 2019. These calves were retained by the owner and were sampled in early summer of 2020. The rest of the calves were born in the spring of 2020 and sampled at the time of weaning in the fall of 2020. Producers were instructed to collect samples from a representative set of at least 20 calves. Statistical randomization within each herd was not performed. If fewer than 20 calves were available, all calves in the group were sampled. All materials for sample collection were provided for the study. If calves were not already individually identified, ear tags were provided. Individual fecal samples were collected prior to treatment and approximately 14 days after treatment from the rectum of each calf using a new sleeve.

Anthelmintic protocols

Producers were instructed to utilize existing anthelmintic application protocols for each operation. The study did not dictate which products were used or how the products were administered. Samples were hand delivered or shipped on ice to the parasitology lab at the Oklahoma Animal Disease Diagnostic Laboratory for analysis.

Herd survey

Participating herds were asked to complete a brief survey describing the type of herd, predominant pasture type and typical grazing management. The survey also asked producers to describe the typical anthelmintic program utilized on the operation and to indicate whether animals were weighed or if weights were estimated.

Laboratory procedures and fecal egg count reduction test

Samples for FEC were evaluated using the Wisconsin method with a limit of detection of 1 egg per gram (EPG). Four grams of feces from each animal were resuspended in 10 ml of Sheather's sugar solution with a specific gravity of 1.25. Feces were thoroughly mixed and passed through a sieve to remove large debris. The slurry was then added to a 15 ml centrifuge tube and extra Sheather's solution was added until a reverse meniscus was formed. A cover slip was placed on top of the reverse meniscus and centrifuged for 5 minutes at 1,500 rpm. Coverslips were then transferred to a slide and all the parasite eggs and oocysts were counted and divided by 4.

For the evaluation of the composite sampling, 1 gram of feces from each animal in the group was combined and mixed 3 different times over a 2-minute period using a tongue depressor. Four grams of the composite sample were then processed using the Wisconsin method as described above to determine the FEC.

A minimum of 25 EPG in the pretreatment sample was utilized as the threshold for inclusion in the study. This threshold was applied at the individual animal level. For the composite samples, all samples in a group were included in the composite sample. Composite samples with at least 25 EPG were included in the final analysis.

Results of individual FECs were reported for each herd as the arithmetic mean of the individual FECs. The fecal egg count reduction (FECR)% was calculated according to the current World Association for the Advancement of Veterinary Parasitology (WAAVP)[9] recommendation, using the following equation: FECR (%) = [1 – (arithmetic mean FEC post treatment/arithmetic mean FEC pretreatment)] x 100%. Fecal egg count reduction of less than 90% was considered indicative of resistance.[8]

Statistical analysis

Approximate 95% confidence limits were calculated for the FECR% based on individual samples according to the methodology described by Levecke.[13] Confidence limits for the composite samples could not be calculated due to the lack of replication in the composite sample analysis.

Lin's Concordance Correlation was calculated to assess the degree of correlation between the FECR% based on the individual samples and the corresponding composite sample.

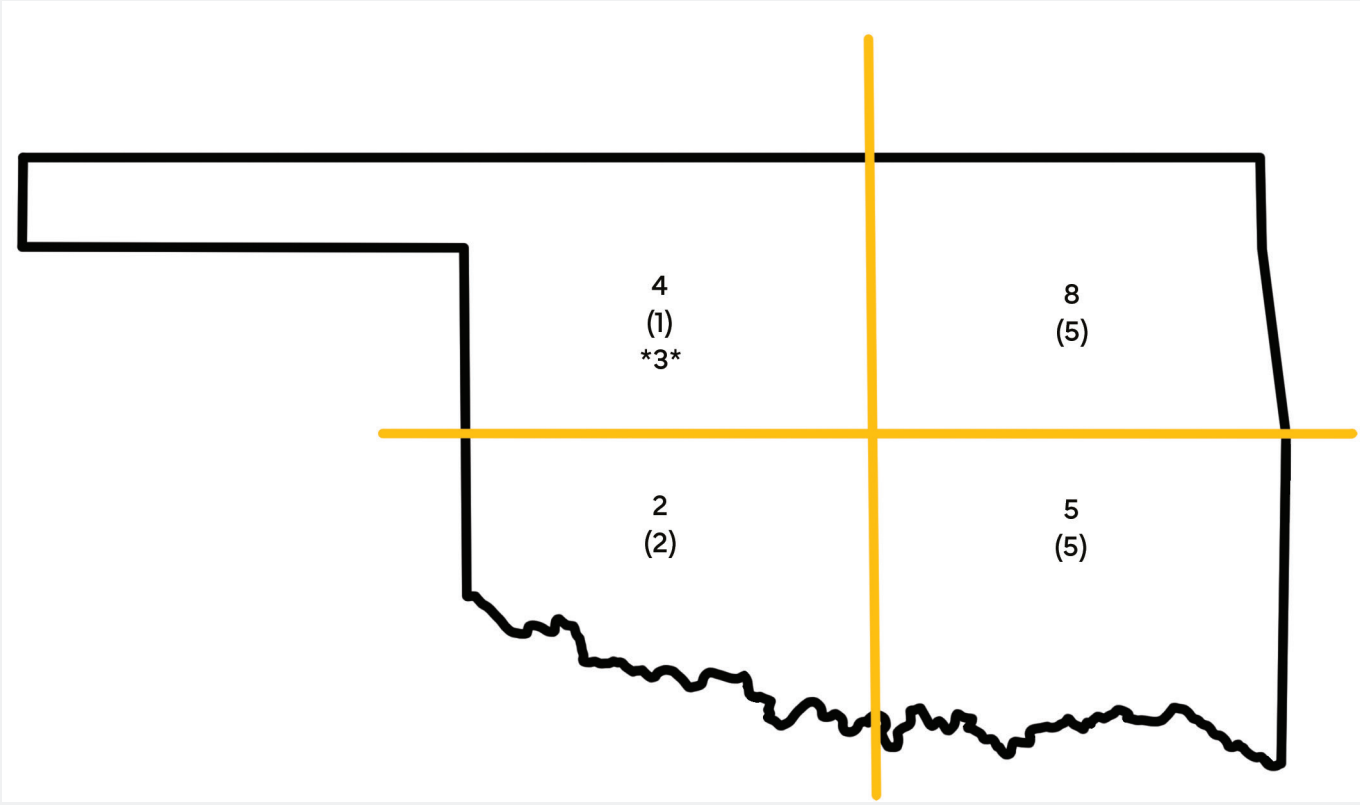
Results

Animals/herds

A total of 17 herds submitted 19 sample sets, with 2 herds submitting 2 different sample sets to evaluate the efficacy of different anthelmintic products within each herd. The number of calves in each sample set ranged from 10 to 29 calves. The state was divided into 4 geographic regions based on the interstate highway system. The number of sample sets from each geographic region were as follows: 4 from the northwest, 8 from the northeast, 5 from the southeast, and 2 from the southwest (Figure 1).

Based on the 25 EPG pretreatment threshold for inclusion in the final analysis, 3 sample sets from 3 different herds were excluded because none of the calves in these sample sets met the 25 EPG minimum. All of these herds were from the

Figure 1: Distribution of sample sets submitted for FECRT.



Top number indicates the total number of sample sets submitted from each region.
() indicates the number of sample sets with apparent resistance.
** indicates the number of sample sets excluded from the final analysis due to inadequate pretreatment FEC.

northwest region of the state. The number of calves excluded from the other sample sets ranged from 0-10 so that the number of calves remaining in each set for the final analysis ranged from 8-24 calves. The 16 sample sets in the final analysis represented herds from all geographic regions of the state. Three of the 4 herds from the northwest part of the state were excluded due to inadequate initial FEC.

Herd surveys

All herds participating in the study submitted survey results, although not all surveys were complete. Of the herds included in the final analysis, 12 herds indicated that they were commercial herds while 2 were seedstock herds and 2 were a combination of commercial and seedstock. In 4 herds, the predominant pasture type was native grass while 7 herds grazed predominantly improved pastures and 5 herds grazed a combination of native and improved grasses. Seven herds practiced continuous grazing strategies, 7 herds practiced rotational grazing and 2 herds indicated a combination of continuous and rotational grazing management practices. Stocking rate was variable depending on the type of grazing management used and the geographic location of the herd. Twelve herds stated that deworming adult cattle at least once per year was part of routine management while only 1 herd stated clearly that adult cattle were not routinely dewormed. The remaining herds did not clearly indicate whether adult cattle were routinely dewormed. Seven herds indicated that anthelmintic products were dosed based on measured individual animal weights whereas the remaining herds calculated anthelmintic doses based on estimated weights.

Anthelmintic products

A total of 8 anthelmintic products were used by herds participating in the study. Those anthelmintic products along with the number of herds using each were: moxidectin pour-on[b] (5), doramectin injection[c] (2), doramectin pour-on[d] (4), generic ivermectin injection[e] (2), generic ivermectin injection[f] (1), generic ivermectin pour-on[g] (1), fenbendazole drench[h] (3), and albendazole drench[i] (1).

Fecal egg count reduction tests

The FECRT failed to reach the 90% threshold for effectiveness in 13/16 (81%) sample sets included in the final analysis. For these 13 sample sets, the percent reduction ranged from -46% to 72% (Table 1). Three sets (19%) of cattle achieved acceptable FECR. The percent reduction in these sample sets were all ≥98%. The 3 sample sets that achieved adequate FECR all used fenbendazole oral drench solution[e] and represented herds in the northeastern region of the state. As used in this study, all other anthelmintic products failed to achieve adequate FECR. Evidence of resistance based on FECR < 90% occurred in herds representing all 4 geographic regions of the state.

A total of 13 sample sets were included in the analysis of composite samples. The 3 sample sets that were excluded from the individual analysis were also excluded from the composite analysis (Table 1). Two of the 13 sample sets achieved FECR > 90% based on the composite samples. Those 2 sample sets also had FECR > 90% based on the arithmetic means of individual samples. Fecal egg count reduction based on the composite

Table 1: Fecal egg counts and fecal egg count reduction percentage for individual and corresponding composite samples.

Number of calves					Arithmetic mean FEC					Composite sample FEC		
Group ID	Total	> 25 EPG	Region	Anthelmintic product	Pre-treat FEC	Post-treat FEC	FECR%	Lower 95% CL	Upper 95% CL	Pre-treat FEC	Post-treat FEC	FECR %
4	20	12	NW	Cydectin PO	65.6	28.3	56.8%	28.8%	73.8%	< 25 EPG		
10	10	8	SW	Cydectin PO	106.0	46.9	55.7%	-20.5%	83.7%	80.0	35.3	55.9%
1	17	17	NE	Dectomax Inj	605.9	538.4	11.1%	-124.6%	64.8%	NA		
13	20	17	SE	Dectomax Inj	389.6	314.7	19.2%	-15.2%	43.4%	271.5	146.0	46.2%
11	29	24	NE	Dectomax PO	191.8	207.3	-8.0%	-104.6%	42.9%	90.5	28.5	68.5%
16	20	19	SE	Dectomax PO	105.9	42.2	60.1%	35.5%	75.3%	69.5	63.8	8.3%
17	22	20	NE	Dectomax PO	423.3	276.7	34.6%	2.7%	56.1%	556.8	385.5	30.8%
18	20	16	SE	Dectomax PO	158.2	50.9	67.8%	40.1%	82.7%	89.0	22.0	75.3%
6	20	17	NE	Noromectin	89.3	67.1	24.9%	-32.1%	57.2%	37.5	66.8	-78.0%
8	20	10	NE	Noromectin	53.2	35.8	32.8%	-50.2%	69.9%	30.0	12.5	58.3%
19	20	17	SW	Ivermax Inj	192.9	54.0	72.0%	37.0%	87.6%	180.8	57.5	68.2%
12	18	14	NE	Bimectin PO	98.5	144.3	-46.5%	-144.1%	12.0%	121.5	201.0	-65.4%
7	16	15	NE	Safeguard Oral	73.5	0.9	98.8%	97.4%	99.4%	< 25 EPG		
9	20	11	NE	Safeguard Oral	745.0	0.7	99.9%	99.3%	100.0%	26.5	0.5	98.1%
14	20	10	NE	Safeguard Oral	72.6	0.3	99.6%	99.2%	99.3%	47.3	0.0	100.0%
15	20	15	SE	Valbazen Oral	74.0	27.0	63.5%	-80.1%	92.6%	84.3	16.8	80.1%

samples ranged from -78% to 100%. There was considerable variation between the FECR% of the individual samples and the FECR% for the corresponding composite sample. The value for the Lin's concordance correlation was 0.665 (95% CI 0.269-0.869). Despite the variation in FECR% between individual and the corresponding composite samples, there was 100% agreement between the individual and composite samples in terms of detecting evidence for resistance at the herd level. The third herd that demonstrated anthelmintic efficacy based upon individual FEC was excluded from composite sample assessment due to pretreatment FEC < 25).

Discussion

Anthelmintic resistance in beef cattle is a growing concern across the U.S. To date, very few studies have evaluated the level of resistance within a specific state or geographic region. Cattle movement increases the risk of resistance spreading across the country but environmental and management differences may result in differences in resistance risk for various geographic areas. Investigation of anthelmintic resistance in U.S. beef herds from multiple states was conducted as part of the 2007-2008 NAHMS Beef Cow-Calf Survey.[8] This survey included 4 herds from Oklahoma, 2 of which had evidence of anthelmintic resistance. This study provided evidence that anthelmintic resistance does exist in beef cattle in Oklahoma, but it is impossible to assess the prevalence and distribution of resistance on a broader scale due to the small number of herds involved and the enrollment process used. The purpose of the survey reported here was to explore the prevalence and distribution of anthelmintic resistance in beef cattle herds in Oklahoma.

This study represents a broad assessment of anthelmintic efficacy in Oklahoma beef cattle herds. Although the number of participating herds was relatively small, the herds were dispersed throughout the state giving the study broad geographic relevance within the state. Participating herds included both seedstock and commercial cow-calf herds. Herds grazing both native and improved pastures are represented as are herds utilizing both continuous and rotational grazing management systems. The small number of herds and the lack of anthelmintic efficacy in the majority of herds prevented any analysis to determine the impacts of herd type, pasture type or grazing management strategy on the prevalence of anthelmintic resistance.

Based on arithmetic means of individual samples, the threshold of ≥ 90% reduction in FEC was only achieved in 3 groups of calves in this study indicating a very high rate of apparent resistance to commonly used anthelmintic medications. This high rate of apparent resistance was a surprise to the study authors and is very concerning. Apparent resistance was not isolated to a particular herd type, pasture type or grazing management strategy. Likewise, the 3 groups of cattle that achieved adequate FECR represented both native and improved pastures

and continuous and rotational grazing systems. Based on the results of this study, apparent anthelmintic resistance is a concern for producers throughout the beef industry in Oklahoma regardless of herd type, pasture type or grazing system used.

There is variation in the published literature regarding the most appropriate threshold for detecting the presence of anthelmintic resistance using FECRT. The threshold of 90% reduction used in the current study is consistent with other recent published studies.[8,14-16] The WAAVP guidelines have historically recommended a threshold of FECR% < 95% with the lower limit of the 95% confidence interval < 90%.[9] Several recent publications have used this methodology as well.[13,17,18] However, a recent update to the WAAVP guidelines recommends use of FECR% < 90% as the threshold for detecting reduced efficacy.[19] For the data reported here, the use of either definition of resistance results in the same classification of the parasite populations regarding the presence or absence of apparent resistance (Table 1).

The use of the FECRT to assess and monitor anthelmintic efficacy has not been widely adopted in the beef industry. There are probably many reasons for this but one of the most notable is likely the cost of analyzing large numbers of samples. Collection and analysis of samples from 17-20 calves has been recommended for accurately assessing herd level anthelmintic effectiveness[8] leading to significant expense for sample analysis. Combining these samples into a pooled or composite sample for FECR testing is one potential way to greatly reduce the expense of testing. The use of composite sampling has been used to estimate individual FEC[11] and to assess FECR[10] in sheep. Only one study[12] has reported the results of the use of composite samples for FECR testing in cattle. This study included 14 groups of cattle and reported a high level of agreement and correlation between FECR% based on individual samples with FECR% based on composite samples (Lin's Concordance Correlation Coefficient 0.9586, 95% CI 0.8700 - 0.9872). The classification of a population as susceptible or resistant was consistent for all 14 groups of cattle for both sampling methods. A secondary objective of the study reported here was to conduct a similar assessment of composite sampling for FECR testing. Lin's Concordance Correlation in the current study (0.665, 95% CI 0.269 - 0.869) was lower than the corresponding value reported by George.[12] One possible explanation for the lower correlation found in the current study could be slight differences in how the correlation was calculated. George et al. excluded negative FECR% values from the correlation calculation. Negative FECR% values were not excluded in the study reported here due to the already small number of values included in the calculation. Despite the lower correlation coefficient found in the current study, perfect concordance was found between FECR testing using arithmetic means of the individual samples and the FECR testing using composite samples for herd level detection of resistance. When the results of this study are considered in light of the findings from George et al.[12] it appears as though FECR testing using composite samples warrants further investigation as a way to reduce cost and possibly increase adoption of FECR testing for assessing anthelmintic efficacy in beef cattle operations.

Although a Wisconsin method with a sensitivity of 1 EPG was used for the FEC, the authors have concerns about the low pretreatment FEC in some of the composite samples and 2 herds were excluded from the composite sample analysis because their pretreatment composite samples had < 25 EPG. These low FECs could possibly impact the repeatability of the findings of this study.

Apparent resistance was identified to multiple classes of anthelmintic products including multiple avermectins, moxidectin and a benzimidazole. The findings are consistent with other reports that have identified resistance to a wide array of anthelmintic products.[5-7] It is important to note that the resistance to a wide array of products found in this study does not necessarily provide evidence of multi-drug resistant parasite populations. Herds in this study only administered a single anthelmintic product so the effects of combination therapy or the presence of multi-drug resistance could not be evaluated. The findings indicate that resistance is not isolated to a single or even a few isolated anthelmintic products. The finding that oral fenbendazole was the only effective anthelmintic product used in this study should be interpreted with caution. In all 3 of the herds that achieved acceptable FECR, fenbendazole was a novel product. Two of these herds had used injectable ivermectin for many years. In these 2 herds, 1 set of calves was treated with injectable ivermectin while a second set of calves was treated with oral fenbendazole. In both of these herds, the ivermectin product failed to achieve adequate FECR while fenbendazole resulted in FECR > 98%. Fenbendazole had not been used on either of these farms in many years. The third herd had used a variety of products over the last several years but had just started using fenbendazole within the last year. Had other products been used under the same circumstances of novelty, the observed results may have been different.

Based on the results of the FECR survey conducted as part of the NAHMS Beef Cow-Calf 2007-08 survey,[8] it appears that resistance may be more common when herds use pour-on anthelmintic products compared to injectable or oral formulations. The fact that the majority of the groups of cattle that participated in the current study had apparent resistance prevented any meaningful comparison of the efficacy of different types of anthelmintic products.

The term apparent resistance should be used when discussing the results of this study because factors other than true resistance may have played a role in the results observed. This study did not control the anthelmintics used or how those anthelmintic products were administered. Nine of the groups of cattle included in the final analysis were dosed based on estimated weights while 7 were dosed on measured individual weights. Estimating weights can easily lead to under-dosing which may contribute to apparent resistance. Of the 7 groups that were weighed, 4 groups still had evidence of apparent resistance so it is unlikely that under-dosing due to estimating weight is the primary cause of the apparent resistance observed. Many factors related to product storage, handling and administration can impact the overall effectiveness of the product. None of those factors were controlled in this study.

Based on the results of this small survey, it appears that apparent anthelmintic resistance is widespread in beef cow-calf herds in Oklahoma. While other uncontrolled factors may have contributed to the observed apparent resistance, it is unlikely that those factors explain all of the observed findings. Regardless of whether these findings indicate true resistance or some combination of true resistance and other factors, this study indicates clearly that many beef producers in Oklahoma are not getting the benefit from their anthelmintic control programs that they expect. Conducting periodic FECR tests is the best way to assess and monitor anthelmintic efficacy. Performing FECRT using composite samples may prove to be an effective way to detect resistance at the herd level while reducing the

costs of testing. Veterinarians should encourage producers to adopt FECR testing as part of routine parasite management practices while encouraging producers to adopt sustainable nematode control practices.

Endnotes

[a]**https://www.aphis.usda.gov/animal_health/nahms/beefcowcalf/downloads/beef0708/Beef0708_dr_PartIV_1.pdf** (Accessed April 19th, 2022)

[b]Cydectin® pour-on, Elanco Animal Health, Greenfield, IN

[c]Dectomax® injectable solution, Zoetis, Parsippany-Troy Hills, NJ

[d]Dectomax® pour-on, Zoetis, Parsippany-Troy Hills, NJ

[e]Noromectin® injection, Norbrook, Overland Park, KS

[f]Ivermax® injection, Aspen Veterinary Resources, Liberty, MO

[g]Bimectin® pour-on, Bimeda, Overbrook Terrace, IL

[h]Safeguard® oral solution, Merck Animal Health, Kenilworth, NJ

[i]Valbazen® oral solution, Zoetis, Parsippany-Troy Hills, NJ

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Conflict of interest

The authors have no known conflicts of interest to declare.

Author contributions

Dr. John Gilliam was the primary author and managed logistics of the study including sample collection and submission, recruitment of cooperating personnel, and manuscript preparation. Dr. Jared Taylor assisted with study design, manuscript editing and conducted all data analysis. Dr. Ruth Scimeca assisted with study design, manuscript editing and conducted or oversaw all laboratory procedures for the study.

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